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A New HPLC-ESI-MS/MS Method to Characterize and Quantify Phosphatidyl-Choline With VLC-PUFA: Application to Human Retina

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Abstract

Purpose: Mutations in the *ELOVL4* gene have been found in Stargardt-like macular dystrophy or STD3. Previous studies have shown that ELOVL4 is involved in the biosynthesis of very long chain polyunsaturated fatty acids (VLC-PUFA). The aim of this work was to develop a HPLC-ESI-MS/MS method of characterization and quantification of dipolyunsaturated phosphatidyl-choline (PC) molecular species containing VLC-PUFA and to apply it on retinas from human donors.

Methods: Eyeballs were collected from calf as well as from nine human donors (body donation to Science). The neural retina was dissected from the RPE/choroid. Following lipid extraction, phosphorus content of total phospholipids was determined.Using a triple quadrupole MS instrument, PC molecular species were structurally characterized by collision-induced dissociation in the negative mode with a method based on normal-HPLC-ESI-MS/MS. PC molecular species were then quantified using precursor ion scanning of m/z 184amu in the positive mode.

Results: The characterization of PC species was done on bovine retinas. Among them, 28 were dipolyunsaturated PC species containing one VLC-PUFA (C24 to C36) with three to six double bonds. VLC-PUFA were always in the *sn*-1 position whilst PUFA at the *sn*-2 position was exclusively docosahexaenoic acid (DHA, C22:6.n-3). Most of these VLC-PUFA-containing dipolyunsaturated PC were detected and quantified in human retinas. The main represented compounds were those having VLC-PUFA of 32 carbon atoms (C32:3, C32:4, C32:5 and C32:6) and 34 carbon atoms (C34:3, C34:4, C34:5 and C34:6). Dipolyunsaturated PC with 36:5 and 36:6 were detected in lower quantities.

Conclusions: This new HPLC-ESI-MS/MS method is sensitive and specific enough to structurally characterize and quantify all molecular species of PC, including those esterified with VLC-PUFA. This technique is valuable for a precise characterization of PC containing VLC-PUFA in retina and may be useful for better understanding their implication in the pathogenesis of STD3.

Keywords: lipids • retina • clinical laboratory testing